# MICROBIAL PROTEIN PRODUCTION BY *PSEUDOMONAS AERUGINOSA* USING SAWDUST SUBSTRATE IN SUBMERGED FERMENTATION

**Nwokoro,** Ogbonnaya[1]

[1]Department of Microbiology, University of Nigeria, Nsukka, Nigeria

E-mail address: ogbonnaya.nwokoro@unn.edu.ng

***Abstract***

*The effects of various substrates and their concentrations on the production of microbial protein by Pseudomonas aeruginosa were investigated at flask level. Different concentrations of sawdust were added into media containing varying amounts of sterile distilled water and inoculated with Pseudomonas aeruginosa culture and incubated. Bacterial biomass of 5.2 x $10^8$ cfu/ml and best protein yield of 3.9 mg/ml were produced with sawdust concentration of 6.5g and distilled water concentration of 93.5 ml. When the substrate concentration was decreased from 6.5 g to 0.5 g, there was a substantial decrease in bacterial biomass and crude protein production. When ammonium sulphate was tested for its effect on protein production in media with the same sawdust concentration, it was observed that control sample which was devoid of ammonium sulphate gave the lowest amount of protein (1.9 mg/ml). The best protein yield of 6.9 mg/ml was obtained when ammonium sulphate concentration was 0.5 g but increasing its concentration to 0.6g, resulted in the production of lower amount of protein. The addition of dried goat faecal matter into the fermentation broth revealed that protein production increased with increase in the concentration of dried goat faecal content. At a level of 25 g faecal matter, the overall highest yield of protein (18.3mg/ml) was obtained whereas, at the lowest goat faecal matter content of 5 g, only 6.5 mg/ml of protein was obtained. This work has shown the biotechnological potential of this bacterium and its ability to grow in sawdust and accumulate protein.*

**Keywords:** Sawdust, microbial protein; *Pseudomonas aeruginosa*; submerged fermentation.

Received: 06.08.2020 Reviewed: 09.01.2021 Accepted: 27.01.2021

## INTRODUCTION

One of the earth's major renewable resources is wood and this bioresource is formed from three main polymeric materials namely, cellulose, lignin and hemicelluloses (Tanaka et al., 2009). All over the world, fallen wood stores more than 73 billion tones of carbon (Pan et al., 2011) and also it serves as a habitat for many saproxylic i.e dead wood-inhabiting organisms (Stokland et al., 2012). Among the main organisms involved in wood decomposition are fungi but bacteria also live in dead wood (Johnston et al., 2016). Cellulose and lignin are among the most abundant polymers in nature and are present in the cellular cell wall conferring structural support, impermeability and resistance against microbial attack and oxidative stress (Parez et al., 2002). Natural lignocelluloses are derived from wood, grass, agricultural residues, forestry wastes etc. Degradation of cellulose, hemicelluloses and lignin has attracted a lot of interests for years because of their economic importance. Materials composed of lignocellulose have been studied as substrates for the production of microbial proteins (Almeida e Silva et al., 1995). Production of microbial protein which is also called single cell protein is an attractive alternative to valorize agro-industrial wastes, municipal wastes and other wastes from wood, paper industries etc. and reducing environmental pollution. Microorganisms that degrade wood are abundantly present in the environment and are found in the terrestrial and aquatic environments. These organisms have developed many mechanisms of adaptation and these enable them to effectively utilize lignocellulose materials and are also able to tolerate extremes of environmental conditions.

Sawdust is a by product of wood processing and it always regarded as waste and are often burnt or dumped into water bodies. The recalcitrance of wood materials namely cellulose, lignin and hemicelluloses and the importance of their biodegradation by microorganisms have received much attention

(Lennox et al., 2010). Degradation of wood saw dust has resulted in the production of protein, glucose, ethanol etc. (Shide et al., 2004). The degradation rate as well as its product formation depend on the type of microorganisms involved and the environmental conditions under which it occurred.

Microbial proteins have been used over the years to supplement protein – deficient foods and many microorganisms and different substrates are used for their production. Single cell-proteins include dried microbiological cell mass or total extracted protein obtained from pure cultures of microbiological cells such as bacteria, fungi and yeasts. Supplementation of foods including cereals with single cell protein makes them as good as animal proteins (Huang and Kinsella 1986). These proteins which are obtained from microorganisms are cheap and also provide a balanced nutrition for humans and animals (Rajoka et al., 2006). Deficiency of protein is a major problem in the world especially in many developing countries. Increases in human population results in protein and nutrient deficiency. Many efforts have been taken to explore new, alternative and unconventional protein sources for human and animal nutrition since 1950's (Ali et al., 2017). Biomass from microorganisms is thought to be an alternative protein sources to previous conventional sources of food supplements to humans or feed grade food supplements for animals. Microorganisms grow on many varieties of substrates including agricultural wastes and effluents, industrial wastes andthe growth on these materials also help in decomposing pollutants (Huang and Kinsella, 1986). Substrates that are used to produce microbial proteins include substances which contained mono and disaccharides since almost all microorganisms can digest glucose, other hexose and pentose sugars and disaccharides and these materials have a high price tag which puts their economic use for the production of microbial biomass in doubt (Oura 1983). The choice of substances which are renewable and naturally abundant as substrates for microbial growth and protein production offers good attraction from economic viewpoint. Many reports showed that animal excreta have nutritional value and using animal excreta for fermentation provided additional ingredient plus a reduction of environmental pollution (El-Deek et al., 2009). Rumen contents are considered sources of nitrogen and other nutrients for microbial fermentation and these nutrients are utilized without toxicity problems (Khattab et al., 2011). Trichoderma viride is an efficient cellulase producer and *Chaetomium cellulolyticum*, another cellulolytic fungus grew faster and formed 80% more biomass protein than *Trichoderma* (Nasseriet al., 2011). Research has shown the suitability of wood as both carbon and energy source for the growth of fungi and bacteria to produce microbial protein. Single cell protein has also been produced from bacteria including *Brevibacteriu* (Adebayo et al., 2011), *Acinetobacter calcoacenticus, Methylophilus methylitropous, Bacillus megaterium, Acromobacter delvaevate, Bacillus subtilis* (Gomashe et al., 2014), *Aeromonas hydrophilla, Cellulomonas sp., Methylomonas methylotrophus, Thermomonospora fusca, Flavobacterium species, Pseudomonas fluorescens, Rhodopseudomonas capsulata* (Dhanasekaran et al., 2011). This work is aimed at the isolation of bacteria from forest wood samples undergoing decomposition and to use the selected isolate to degrade ground sawdust and produce microbial protein.

## MATERIAL AND METHODS

### Substrate and organism

Wood samples were collected from fallen forest trees which were undergoing microbial decomposition. The sample ca. 10 g was collected into conical flasks and taken immediately to the laboratory. Into the flasks were added 50 ml of sterile Nutrient broth (Oxoid, UK). This was followed by incubation in a Gallemkamp orbital shaker at 50xg for 24 h. The sample was serially diluted and plated on Nutrient agar medium containing chloramphenicol solution to inhibit fungal contaminants. Petri plates were inoculated with diluted samples and incubated for 24 h at 35$^{o}$C.

Pure bacterial colonies were picked and given arbitrary code numbers and stored in slants at 4°C.

Dried sawdust samples were collected, milled to fine powder and sieved with sieve cloth. In a preliminary experiment, 20 g of the milled saw dust sample was added into flasks containing mineral salt medium which had the following composition: $KH_2PO_4$, 0.3g, $K_2HPO_4$ 0.1g, Yeast extract 0.5g and distilled water to 100ml (pH=6.1). The flasks were each inoculated with pure bacterial isolates and incubated with shaking for 48 h after which the contents of the flasks were each harvested by centrifugation at 5000xg, and washed with sterile distilled water and dried at 105°C. Total protein content wasestimated by the method of Lowry et al., (1951) using bovine serum albumin as standard. Out of a total of eleven isolates, the isolate with the code numberB9 produced the highest amount of protein and was therefore identified as *Pseudomonas aeruginosa* based on its cultural, biochemical and physicochemical characteristics as outlined in Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

**Effects of concentrations of milled sawdust samples on protein production**

Conical flasks contained different concentrations of saw dust (0.5, 1.5, 2.5, 3.5, 4.5, 5.5 and 6.5g) in varying amounts of sterile distilled water. Into the flasks were added mineral salt medium with same content as described above. Flasks were inoculated with $2.5x10^5$ colony forming units of *Pseudomonas aeruginosa* and incubated at 35°C for 48 h with shaking. Fermented product was harvested by centrifugation, dried at 105°C and assayed for total protein content.

**Effects of concentrations of ammonium sulphate on protein production**

Ground saw dust samples (20 g) were each added into flasks containing different concentrations of ammonium sulphate (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6g). A control experiment containing no ammonium sulphate was similarly set up. The experimental flasks were inoculated with cultures of *Pseudomonas aeruginosa* and incubated at 35°C for 48 h with shaking. Fermented product was harvested by centrifugation, dried and assayed for total protein content.

**Effects of concentrations of dried goat faecal matter on protein production**

Fresh goat faeces were collected from farm houses and spread on plastic containers to sun dry. The dried matter was weighed until there was no weight difference. The sample was pulverized using laboratory mortar and pestle and inoculated into flasks containing cultures of *Pseudomonas aeruginosa*, incubated at 35°C with shaking for 48 h. The products were harvested by centrifugation, dried and the total protein content was determined.

## RESULTS AND DISCUSSION

All isolated bacteria were tested for their abilities to produce microbial proteins in media containing ground saw dust samples, yeast extract and buffer solution. After 48 h incubation, the broth culture was centrifuged and the product was dried and assayed for its protein content. The isolate designated B9 produced the highest protein concentration of 2.8mg/ml and was therefore selected for further work (Table 1). This bacterium was identified as Pseudomonas aeruginosa according to the taxonomic descriptions in Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

Different concentrations of sawdust were added into media containing varying amounts of sterile distilled water. The sample was inoculated with Pseudomonas aeruginosa culture. Incubation was done at 24 h intervals for 48 h at 35°C. Results in Table 2 shows that the best bacterial biomass of $5.2 \times 10^8$ cfu/ml and best protein yield of 3.9 mg/ml were produced with sawdust concentration of 6.5g and distilled water concentration of 93.5 ml. When the substrate concentration was decreased from 6.5 g to 0.5 g, there was a substantial decrease in bacterial biomass and crude protein content. Studies showed that the yield of protein depended on the concentration of wood in the medium and in general, higher microbial protein was obtained at highest sawdust levels which corresponded to the lowest amounts of sterile distilled water.

| Isolated bacteria | Protein (mg/ml) |
|---|---|
| B1 | 1.5 |
| B2 | 0.6 |
| B3 | 1.8 |
| B4 | 0.8 |
| B5 | 1.6 |
| B6 | 1.9 |
| B7 | 0.3 |
| B8 | 2.3 |
| B9 | 2.8 |
| B10 | 1.5 |
| B11 | 0.8 |

**Table 1: Protein production by bacterial isolates in preliminary isolation medium**

| Sawdust (g) | 0.5 | 1.5 | 2.5 | 3.5 | 4.5 | 5.5 | 6.5 |
|---|---|---|---|---|---|---|---|
| Distilled water (ml) | 99.5 | 98.5 | 97.5 | 96.5 | 95.5 | 94.5 | 93.5 |
| Viable cells (cfu/ml) | | | | | | | |
| 0 h | $2x10^5$ | $2x10^5$ | $2x10^5$ | $2x10^5$ | $2x10^5$ | $2x10^5$ | $2x10^5$ |
| 24 h | $5.8x10^6$ | $2.6x10^7$ | $11.6x10^7$ | $3.0x10^8$ | $1.6x10^8$ | $3.1x10^8$ | $5.2x10^8$ |
| 48 h | $1.1x10^6$ | $2.3x10^6$ | $1.6x10^6$ | $2.4x10^7$ | $3.1x10^8$ | $3.6x10^7$ | $3.5x10^8$ |
| Protein (mg/ml) | 0.1 | 1.2 | 1.5 | 1.8 | 2.1 | 2.6 | 3.9 |

**Table 2: Production of protein in media with different sawdust concentrations by *Pseudomonas aeruginosa***

The maximum viable cell counts occurred after 24 h incubation (Table 2).

Results on the addition of ammonium sulphate on the rate of protein production by bacterial utilization of sawdust are shown in Table 3. Control sample which was devoid of ammonium sulphate gave the lowest protein of 1.9mg/ml. The best protein yield of 6.9mg/ml was obtained when ammonium sulphate concentration was 0.5 g but at 0.6 g only 4.3 mg/ml protein was obtained (Table 3).

The influence of the addition of dried goat faecal matter on protein production by *Pseudomonas aeruginosa* is shown in Table 4. From the result, it is evident that increasing the concentration of dried goat faecal content to 25g resulted in the overall best yield of protein (18.3mg/ml). At the lowest goat faecal matter content of 5g only 6.5mg/ml of protein was obtained.

| Ammonium sulphate (g/100ml) | 0 | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 |
|---|---|---|---|---|---|---|---|
| Sawdust (g) | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| Protein (mg/ml) | 1.9 | 4.1 | 4.3 | 5.8 | 6.1 | 6.9 | 4.3 |

**Table 3: Effects of different concentrations of ammonium sulphate on protein production by *Pseudomonas aeruginosa***

| | | | | | |
|---|---|---|---|---|---|
| Goat faecal matter (g) | 5 | 10 | 15 | 20 | 25 |
| Distilled water (ml) | 95 | 90 | 85 | 80 | 75 |
| Sawdust (g) | 20 | 20 | 20 | 20 | 20 |
| Protein (mg/ml) | 6.5 | 15.1 | 15.3 | 18.0 | 18.3 |

**Table 4: Effects of different concentrations of dried goat faecal matter on protein production by *Pseudomonas aeruginosa***

The maximum protein production from this work was compared with similar findings. Almeida e Silva et al., (1995) used eucalyptus hemicelluloses hydrolysate as a substrate to produce microbial protein with *Paecilomyces variotii* IOC – 3764. The biomass the authors obtained from this organism had a total protein content of 34%. Chahal et al., (1981) investigated the effects of different pretreatments of aspen wood for the production of single cell protein using *Chaetomium cellulolyticum* and they reported that high pressure steam was superior to atmospheric pressure steam for the breakdown of wood. Increased protein composition in the final product was 37.9%. Wang et al., (2001) reported that steam exploded corn stalks produced crude protein of 31.82% when cultured with *Trichoderma reesei* and *Candida* in mixed culture fermentation. Ammonia pretreated corn stalks produced crude protein of 18.13% with *Trichoderma reesei, Aspergillus niger* and *Candida tropicalis* (Chen et al., 2000). NaOH pretreated rice straw produced a crude protein yield of > 50% with *Candida tropicalis* 321 and 181 (Benerjee et al., 1995). A protein yield of 26.02% was reported by Wu and Ma (2002) who used *Candida utilis* and *Rhizopus nigricans* as strains and milled sugar cane bagasse as substrate. Alkali pretreated bagasse yielded a crude protein concentration of 21 – 28% with *Trichoderma sp.* and *Aspergillus terreus* (El-Nawwi and El-Kader 1996). Rajoka et al., (2004) investigated the production of microbial protein from defatted rice polishing using *Candida utilis* and the authors reported biomass yield of 0.62g cells/g substrate and crude protein yield of 27.8%. Rao et al., (2010) reported 46% microbial protein produced by the cultivation of *Penicillium janthinellum* in a medium which contained bagasse hydrolysate, ammonium sulphate and potassium dihydrogen phosphate. Miller and Srinivasan (1983) reported 23 – 38% microbial protein content when they cultivated *Aspergillus terreus* on cellulose substrate. A maximum protein yield of 18.25% was obtained by Khan and Dahot (2010) who used rice husk as carbon source to cultivate *Penicillium expansum*.

## CONCLUSION

This work studied some culture conditions for microbial protein production by *Pseudomonas aeruginosa* using sawdust substrate in growth media. Levels of protein production generally depended on the amounts of sawdust in media. Ammonium sulphate concentrations were used to test their effects on microbial protein production. Yields increased until ammonium sulphate level of 0.6 g which resulted in lower protein production. Goat faecal matter greatly improved protein production and the overall best protein yield was observed at its highest concentration in culture medium.

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